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Protein Metabolism in Lecithotrophic Larvae (Gastropoda: *Haliotis rufescens*)

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Abstract. Rates of protein depletion, synthesis, and turnover were measured in larvae of the abalone *Haliotis rufescens* as an approach to understanding macromolecular metabolism during lecithotrophic development. Protein content decreased linearly during development to metamorphic competence, with 34% of the initial protein in eggs depleted during the 8-day larval life span. Fractional rates of protein synthesis (percentage of total body-protein synthesized per day) decreased during development, from 40% (1-day-old trochophore larva) to 14% (7-day-old veliger larva). Separation of proteins by one-dimensional gel electrophoresis showed that protein pools in larvae are dominated by two high-molecular-weight protein classes (88 and 121 kDa). When the proteins of 1- and 3-day-old larvae were labeled with a mixture of ^{35}S-methionine and cysteine, the pattern on two-dimensional gels showed that the turnover process (protein synthesis and degradation) involved hundreds of different proteins. The energy gained from loss of protein could account for 20% of the protein turnover rates for trochophore larvae and 79% of the lower turnover costs for late-stage veligers. Lecithotrophic larvae of *H. rufescens* maintained high biosynthetic activities, with up to 40% of their whole-body protein being turned over each day. Such dynamic processes during development of nonfeeding larvae would contribute significantly to maintenance metabolism.

Introduction

Nonfeeding (lecithotrophic) larval forms are widely represented in the life cycles of many marine invertebrate taxa (Thorson, 1950; Strathmann, 1978; Pearse, 1994). Nevertheless, our understanding of metabolic processes during lecithotrophic larval development is very limited compared to what is known about metabolism and the dynamics of biosynthetic processes in species with planktotrophic development (*e.g.*, protein synthesis in sea urchin embryos: Goustin and Wilt, 1981; Bedard and Brandhorst, 1983). Most studies of the biochemistry and physiology of lecithotrophic larvae have focused on the composition of energy reserves in the early stages of development (Turner and Rutherford, 1976; Jaeckle and Manahan, 1989a; Anger, 1996). The importance of maternally endowed energy supply to larval life span has also been studied (Shilling and Manahan, 1994; Ben-David-Zaslow and Benayahu, 1998), as have respiration rates and amino acid transport (Jaeckle and Manahan, 1989a, b; Shilling *et al.*, 1996; Hoegh-Guldberg and Emlet, 1997).

The aim of this study is to quantify the energetics of protein degradation and synthesis (turnover) in a lecithotrophic larval form. Protein metabolism was chosen as the focus of this study because protein, not lipid, has been reported (Jaeckle and Manahan, 1989a) to be the major endogenous energy reserve utilized by larvae of *Haliotis rufescens* (red abalone), a species with lecithotrophic development.

Materials and Methods

Larval culture

Adult abalone (*Haliotis rufescens*) were spawned and fertilized at a commercial hatchery (Ab Lab, Port Hueneme, California). All cultures were started using gametes from 1 male and 2–3 females (different adults were used for each culture). Zygotes and larvae were maintained at 14–15°C in unstirred, UV-irradiated seawater that had been passed through a 5-μm (pore size) filter; the larvae were reared on

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80-μm mesh screens. Under these culturing conditions, it took 6–8 days for larvae to reach metamorphic competence, as determined by ability to undergo metamorphosis when induced with γ-aminobutyric acid (GABA).

Change in total protein

Throughout development, samples (Culture 1, n = 4–5 independent samples; Culture 2, n = 3 independent samples) containing 50–75 individuals were taken for measurement of protein content (Bradford assay, 1976; as modified by Jaeckle and Manahan, 1989a). The rate of protein loss during development was calculated from the linear regression of total protein per individual with time, during the period from egg to metamorphic competence. Protein content of eggs (day zero) was determined only for Culture 1.

Rates of amino acid transport

To determine absolute rates of protein synthesis, rates of amino acid transport by larvae were measured during the ^{14}C-labeling experiments (see Manahan, 1983, for methods). All physiological rates were measured at 15° ± 0.1°C. Each transport assay used 5000 larvae in 10 ml of filtered (pore size 0.2 μm) seawater to which was added 1 μCi ml^{-1} of ^{14}C-(U)-glycine (New England Nuclear; 110 μCi μmol^{-1}). The rate of isotope transport was measured (500 μl sample every 2–3 min) during a 16–18-min exposure of larvae to 9 μM glycine in seawater.

Rates of protein synthesis

A modified radioisotope-labeling protocol, based on that of Fry and Gross (1970) for sea urchin embryos, was used to measure absolute rates of protein synthesis in abalone larvae. Our modifications included trace labeling over a short time interval (<18 min) to minimize possible effects of perturbations, caused by high transport rates of exogenous ^{14}C-labeled amino acid, of the free amino acid pool. A short exposure also avoided the interconversion of ^{14}C-glycine, the tracer used in our measurements, into other amino acids (confirmed by analysis with high-performance liquid chromatgraphy, HPLC). Short experiments also minimized any effects from protein degradation (*i.e.*, reutilization of ^{14}C-labeled amino acids due to protein breakdown). Glycine was used as the tracer because it is transported at a high rate and is a measurable fraction of the free amino acid pool in larvae of *H. rufescens* (Fig. 1). These characteristics allowed for accurate HPLC measurement of the change, with time, in the specific activity of glycine in the free amino acid pools of larvae (Fig. 2A). Also, glycine in acid-hydrolyzed, whole-body protein extracts of *H. rufescens* larvae (Table 1) could be measured with HPLC; this value is required for calculation of absolute rates of protein synthesis (see below).

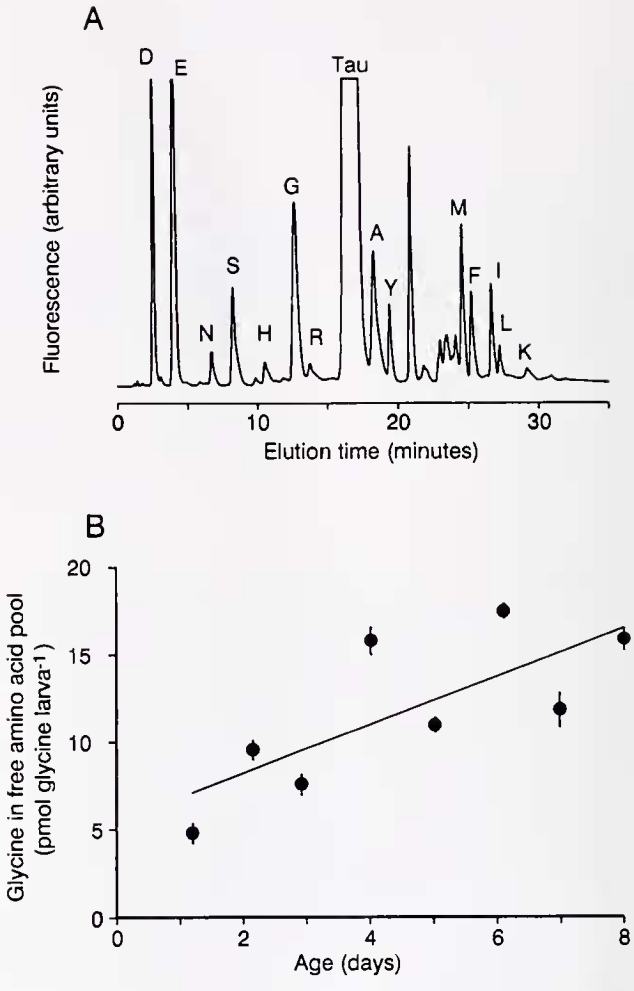

Figure 1. Glycine in the free amino acid pool of larvae of *Haliotis rufescens*. (A) Chromatogram showing the separation of amino acids extracted from 3-day-old veliger larvae by reverse-phase high-performance liquid chromatography: D, aspartic acid; E, glutamic acid; N, asparagine; S, serine; H, histidine; G, glycine; R, arginine; Tau, taurine; A, alanine; Y, tyrosine; M, methionine; F, phenylalanine; I, isoleucine; L, leucine; K, lysine (unknown peaks are unlabeled). (B) Change in the amount of glycine in free amino acid pools through larval development. Error bars represent ± 1 SE of the mean (n = 6 for each stage). The equation for the linear regression is pmol glycine larva^{-1} = 1.51 x + 5.16; where x = age in days (r^2 = 0.50; n = 48).

Rates of protein synthesis were determined by measuring the rate of incorporation of ^{14}C-glycine into protein [defined as the 5% trichloroacetic acid (TCA)-insoluble fraction of larval homogenates, shell included]. For measurements from the free amino acid pools, the intracellular contents of larvae were extracted overnight into 70% ethanol. The specific activity of glycine was then determined with HPLC (Welborn and Manahan, 1995) for each larval stage studied. After detection of fluorescence (*i.e.*, moles of glycine: Fig. 1A, B), post-column samples of eluent were collected every 30 s (LKB fraction collector) and mixed with scintillation cocktail to determine the amount of radioactivity in the

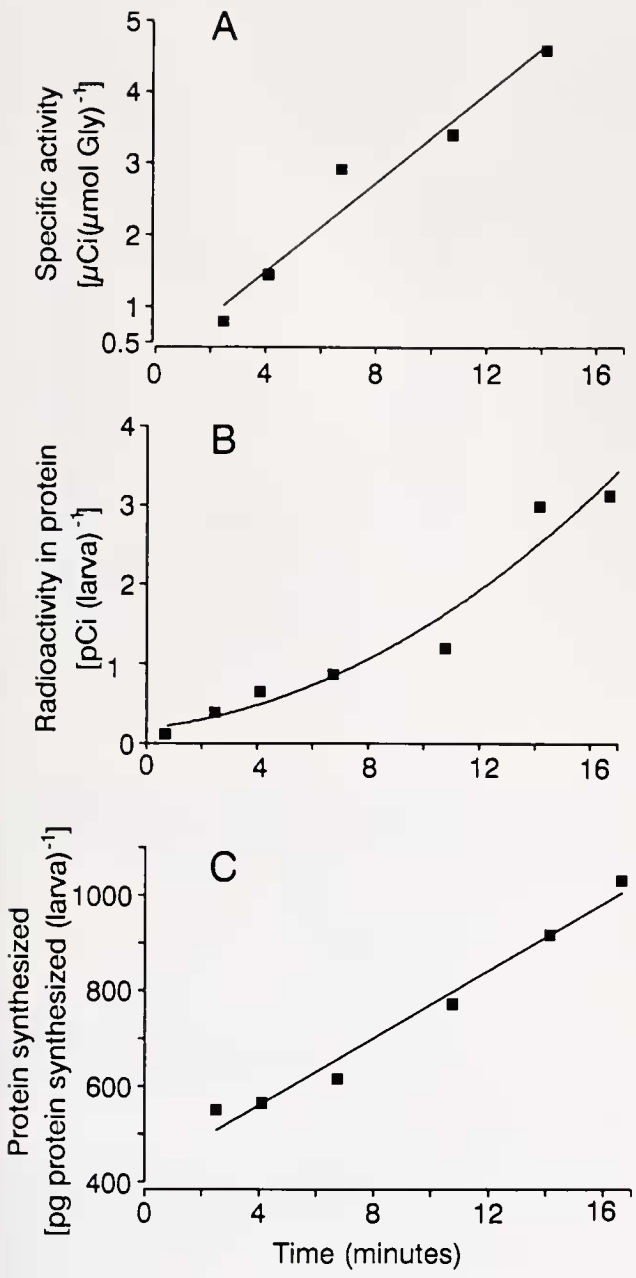

Figure 2. Determination of the absolute rate of protein synthesis in 7-day-old veliger larvae of *Haliotis rufescens*. (A) Specific activity of intracellular glycine in the free amino acid pool following transport of ^{14}C-glycine. Each point represents the specific activity determined by high-performance liquid chromatography using fluorescence detection (moles of glycine) and measurement by liquid scintillation counting of radioactivity in the glycine peak collected from the chromatographic eluent. (B) Radioactivity in the trichloroacetic acid (TCA)-insoluble fraction (protein). (C) Rate of protein synthesis after correcting the TCA-insoluble fraction for the change in intracellular specific activity of ^{14}C-glycine, the mole percent of glycine in protein, and the average mole-percent corrected molecular weight of amino acids in larval protein (Table I).

glycine peak. All measurements of radioactivity (as counts per min, CPM) were corrected for quenching and converted to total disintegrations per min (DPM). By correcting for the change in the specific activity of glycine in the free amino acid pool (Fig. 2A), the incorporation rate of ^{14}C-glycine (Fig. 2B) could be converted to the total amount of glycine incorporated into protein (both ^{12}C- and ^{14}C-glycine). The value for the incorporation of total glycine into protein was then converted to an absolute rate of protein synthesis (Fig. 2C) by determining the mole-percent of glycine in larval protein and the mole-percent-corrected molecular weight of all amino acids in larval protein of *H. rufescens* (Table I). The components required for the calculation of the absolute rate of protein synthesis are

$$k_s = \frac{MW_p}{S_m} \times \frac{d}{dt}\left(\frac{S_p}{S_{faa}}\right)$$

where k_s is the rate of protein synthesis, MW_p is the mole-percent-corrected molecular weight of amino acids in larval protein, S_m is the mole-fraction of glycine in protein, S_p is the amount of radioactivity in protein, and S_{faa} is the specific activity of glycine in the free amino acid pool, which changed during the time course of exposure to isotope (Fig. 2A). The above equation was solved for each sampling time interval during which the incorporation of ^{14}C-glycine into protein was measured (Fig. 2B).

Patterns of protein synthesis

For the analysis of the electrophoretic patterns of protein synthesis, each labeling experiment used 10,000–15,000 larvae in 14 ml of filtered seawater with 200 μCi of ^{35}S-methionine/cysteine (1100–1200 Ci mmol^{-1}, New England Nuclear). Non-radiolabeled methionine was added to seawater (final concentration of 500 n*M*) to increase substrate concentration and, hence, transport rates. After a 3-h incubation, unincorporated isotope was removed with three successive seawater washes after the larvae were pelleted in 15-ml conical tubes with a hand centrifuge. Protein samples for electrophoresis were prepared by ultrasonicating embryos or larvae in 50 m*M* Tris-HCl at pH 7.2. Samples were kept on ice during this process to prevent any rise in temperature. Homogenates were centrifuged to remove larval shell (30 min at 15,000 × *g*, 4°C) prior to electrophoresis. The amount of ^{35}S-methionine/cysteine incorporated into protein was determined by TCA (5%) precipitation of 10-μl aliquots ($n = 2$) of the homogenate. Tissue solubilizer (0.5 ml, Scintigest, Fisher Co.) was added to these precipitates before the radioactivity was counted. These data were required for loading of equal amounts of radioactivity on each gel to permit comparisons of different larval stages (see Results). The amount of protein loaded on each gel was also determined.

Table I

Amino acid composition (mole-percent) of proteins in larval stages of Haliotis rufescens

	Age (days after fertilization)						
Amino acid	2	3	4	5	6	8	Mean (± SEM)
Glutamic acid & Glutamine*	9.9	10.0/10.6	8.6	10.5/10.5	10.7/10.5	10.5	10.2 (0.2)
Alanine	8.4	8.3/8.0	9.0	9.1/8.1	8.1/8.3	8.4	8.4 (0.1)
Glycine	7.0	7.5/7.7	9.9	8.2/8.1	8.0/7.8	8.1	8.0 (0.2)
Aspartic acid & Asparagine*	7.4	7.2/7.3	7.9	6.3/6.4	7.8/7.9	7.6	7.3 (0.2)
Lysine	6.5	6.7/6.5	6.2	6.9/8.1	6.8/7.7	7.4	7.0 (0.2)
Serine	7.0	7.2/7.1	6.3	7.2/7.1	6.9/6.7	7.1	7.0 (0.1)
Leucine	6.8	6.8/6.7	6.8	6.6/7.4	6.6/6.8	6.2	6.8 (0.1)
Valine	6.4	6.9/6.9	6.3	7.2/7.0	6.3/6.2	5.7	6.5 (0.2)
Isoleucine	6.8	6.8/6.7	6.8	3.9/7.4	6.6/6.8	6.2	6.5 (0.3)
Threonine	6.4	5.9/7.0	6.2	6.3/5.4	5.8/5.5	5.6	6.0 (0.2)
Arginine	6.0	6.1/5.7	5.8	6.5/5.8	5.8/5.7	6.2	6.0 (0.1)
Proline	5.9	6.4/5.8	5.2	5.1/5.8	5.0/4.8	4.9	5.4 (0.2)
Tyrosine	4.7	4.0/3.9	5.0	4.0/3.3	4.7/4.6	4.4	4.3 (0.2)
Phenylalanine	3.9	4.0/3.8	3.9	4.0/4.7	4.2/4.2	4.0	4.1 (0.1)
Methionine	3.6	2.8/3.3	3.3	6.1/2.7	3.5/3.3	4.4	3.7 (0.3)
Histidine	2.4	2.6/2.3	1.9	1.7/1.6	2.5/2.4	2.2	2.2 (0.1)
Cysteine	0.8	0.9/0.8	0.8	0.3/0.7	0.8/0.7	1.2	0.8 (0.1)
Mole-percent corrected MW_p†	136.1	135.6/ 135.1	133.7	134.9/ 135.0	136.2/ 136.5	136.8	135.5 (0.3)

All values are calculated as mole percents; where two values are separated by a slash (*e.g.*, 10.0/10.6), this represents two replicate chromatographic analyses for that sample.

* During acid hydrolysis, asparagine and glutamine form aspartic acid and glutamic acid, respectively.

† Mole-percent corrected molecular weights (MW_p) represent the average molecular weight of amino acids in larval protein determined by multiplying each mole percent value by the appropriate molecular weight (*e.g.*, glycine: 0.08 * 75.1 g mol^{-1}), and then taking the sum of all of the mole-percent corrected molecular weights of each amino acid.

Protein extracts of larvae were separated with one- and two-dimensional polyacrylamide gel electrophoresis (PAGE). One-dimensional sodium dodecyl sulfate (SDS)-PAGE was conducted according to Laemlli (1970), with equal amounts of radiolabeled protein (1×10^6 DPM) loaded per lane, totaling about 30 μg protein. The proteins resolved by electrophoresis were stained with Coomassie brilliant blue (Biorad) and were exposed to X-ray film (XOMAT AR, Eastman Kodak) for 24 h. Two-dimensional gel electrophoresis was conducted with pre-cast gels on the Multiphor system (LKB-Pharmacia) following the manufacturer's protocol for the first dimension Immobiline Dry Strip Kit and the second dimension ExcelGel SDS (LKB-Pharmacia: publication 18-1038-63, edition AA). For two-dimensional gels, samples were loaded with equal amounts of radiolabeled protein (3×10^6 DPM), totaling about 100 μg protein per loading. The two-dimensional gels of the different larval stages, each loaded with equivalent amounts of radioactivity, were exposed to X-ray film for an identical time period (8 days).

Results

Change in total protein

There was a linear decrease in protein during development of *Haliotis rufescens* (Fig. 3). The rate of protein loss was not statistically different between Culture 1 (23.7 ± 5.7 ng day^{-1} [± 1 SE of the slope]) and Culture 2 (22.5 ± 5.7 ng day^{-1}); ANOVA: $F_{[1,59]}$ for comparison of slopes = $0.17^{n.s.}$ and for comparison of y-intercepts = $0.17^{n.s.}$. All data were combined to calculate a rate of protein loss from egg to metamorphic competence (nanograms of protein per larva = $-21.2\ x + 498.6$, where x = age in days; SE of slope = 3.52; n = 68). By the end of larval development, 8-day-old larvae still had 66% of their initial (egg) protein content (from regression analysis, Table II).

Glycine transport

Larvae had higher rates of glycine transport near metamorphosis (Culture 1: day 6 = 9.6 pmol glycine $larva^{-1}$ h^{-1}; Culture 2: day 8 = 9.3 pmol glycine $larva^{-1}$ h^{-1}; Fig.

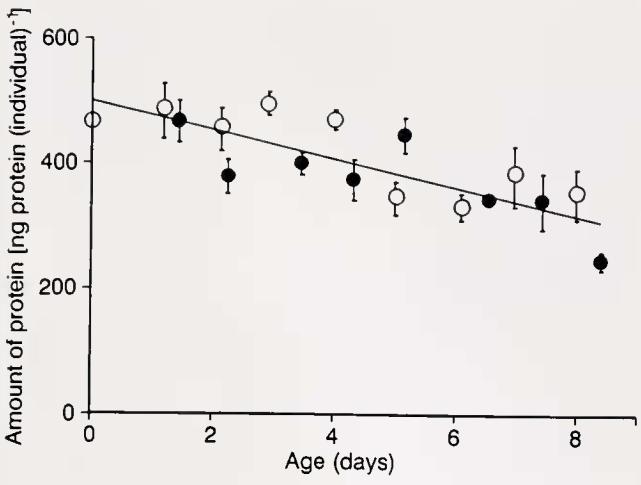

Figure 3. Total protein content during development of *Haliotis rufescens*. Data from two cultures are shown: Culture 1 (n = 4–5 independent samples per data point; ○) and Culture 2 (n = 3 independent samples per data point; ●). Protein content of eggs was determined only for Culture 1. Rates of protein loss were not statistically different between cultures (see text), and all data were combined to calculate the rate of protein loss during development: ng protein individual^{-1} = $-21.2\,x + 498.6$, where x = age in days (SE of slope = 3.52, n = 68). Error bars are ±1 SEM.

4). Combining all glycine transport data for both cultures revealed that transport rates were significantly higher at metamorphic competence (based on larvae before and after day 6; $t_{[24]} = 2.81$, $P < 0.005$).

Table II

Protein synthesis and fractional rates of protein turnover during larval development of Haliotis rufescens

Age (days)	Total protein (ng larva^{-1})	Protein synthesis rate (ng protein synthesized larva^{-1} day^{-1})	Fractional rates of protein turnover (% total protein synthesized day^{-1})
1	477	193	40
2	456	150	33
3	435	115	26
4	414	87	21
5	393	67	17
6	371	54	15
7	350	49	14

Total protein amounts (nanograms of protein per larva) were calculated from the least-squares linear regression of the change in protein content calculated for two different cultures during development (Fig. 3: ng protein larva^{-1} = $-21.2\,x + 498.6$, where x = age in days). Changes in the absolute rate of protein synthesis during development were calculated from the second-order polynomial (Fig. 5: ng protein synthesized day^{-1} = $3.76\,x^2 - 54.09\,x + 243.52$, where x = age in days). Fractional rates of protein turnover (percentage of whole-body protein synthesized per larva per day) were calculated from the ratio of the rate of protein synthesis to total protein content at the corresponding stage of development.

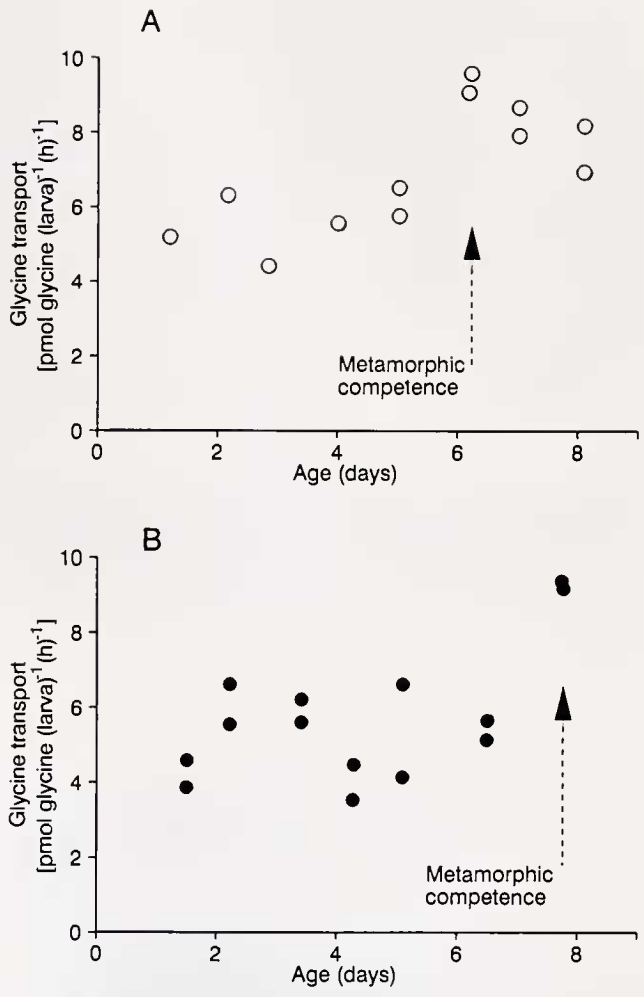

Figure 4. Transport of ^{14}C-glycine (9 μM) from seawater by larvae of *Haliotis rufescens*. Each data point represents the rate of transport calculated from a regression of a separate time-course experiment (4–5 samples per experiment, with about 250 larvae per sample; all assays had r^2 values > 0.96). Data are shown for two cultures: Culture 1 (A) and Culture 2 (B). Arrows show the time when metamorphic competency was reached for larvae from each culture.

Glycine content in free amino acid pools and protein

The amount of glycine in the free amino acid pool increased significantly during development (ANOVA: $F_{[1,46]} = 45.43$, $P < 0.0005$; Fig. 1B). The linear increase in glycine during larval development is represented by the following equation: picomoles of glycine per larva = $1.51\,x + 5.16$, where x = age in days ($r^2 = 0.50$; $n = 48$). Over the larval life span, glycine only represented about 1% of the total free amino acid pool (see Fig. 1A), with taurine being the dominant amino acid. The mole-percent of glycine in protein did not change during development (from glycine data in Table I: ANOVA of the regression slope of protein-glycine over time was not significantly different from zero, $F_{[1,7]} = 0.44^{\text{n.s.}}$). The mean mole-percent of

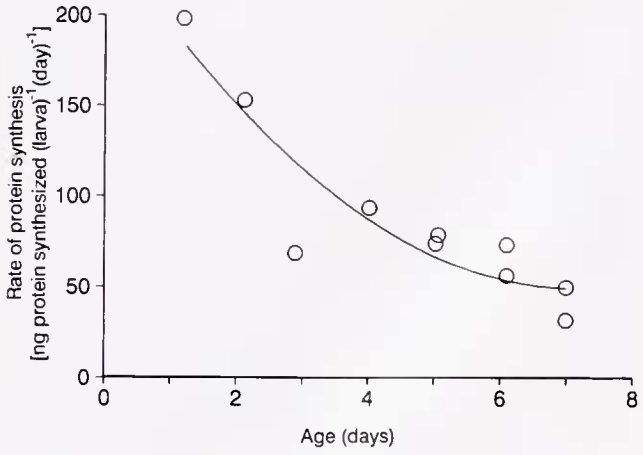

Figure 5. Absolute rates of protein synthesis during larval development of *Haliotis rufescens*. Each data point represents a rate of protein synthesis determined from the slope of a separate time-course experiment, based on multiple samples (as in Fig. 2). The decrease in protein synthesis with development was fitted to a second-order polynomial: ng protein synthesized larva^{-1} day^{-1} = $3.76\,x^2 - 54.09\,x + 243.52$, where x = age in days.

glycine in the proteins of 2- to 8-day-old larvae was 8.0% (±0.2 SEM), and this value was used for all calculations of protein synthesis rates. The mole-percent corrected molecular weight of amino acids in larval protein ranged from 133.7 to 136.8 g amino acid mol^{-1}, with a mean value of 135.5 (±0.3, SEM) used for all calculations (Table I).

Rates of protein synthesis

Rates of protein synthesis decreased during development, from 193 to 49 ng protein synthesized larva^{-1} day^{-1} (calculated from analysis given in Table II of data shown in Fig. 5). The decrease was nonlinear and was best described by a second-order polynomial (nanograms of protein synthesized per larva = $3.76\,x^2 - 54.09\,x + 243.52$, where x = age in days). The fractional rate of protein synthesis, expressed as the percentage of total whole-body protein synthesized per day, also showed a steady decline through larval development and decreased from 40% (1-day-old) to 14% (7-day-old) (Table II: comparison of total protein content per larva and absolute rate of protein synthesis).

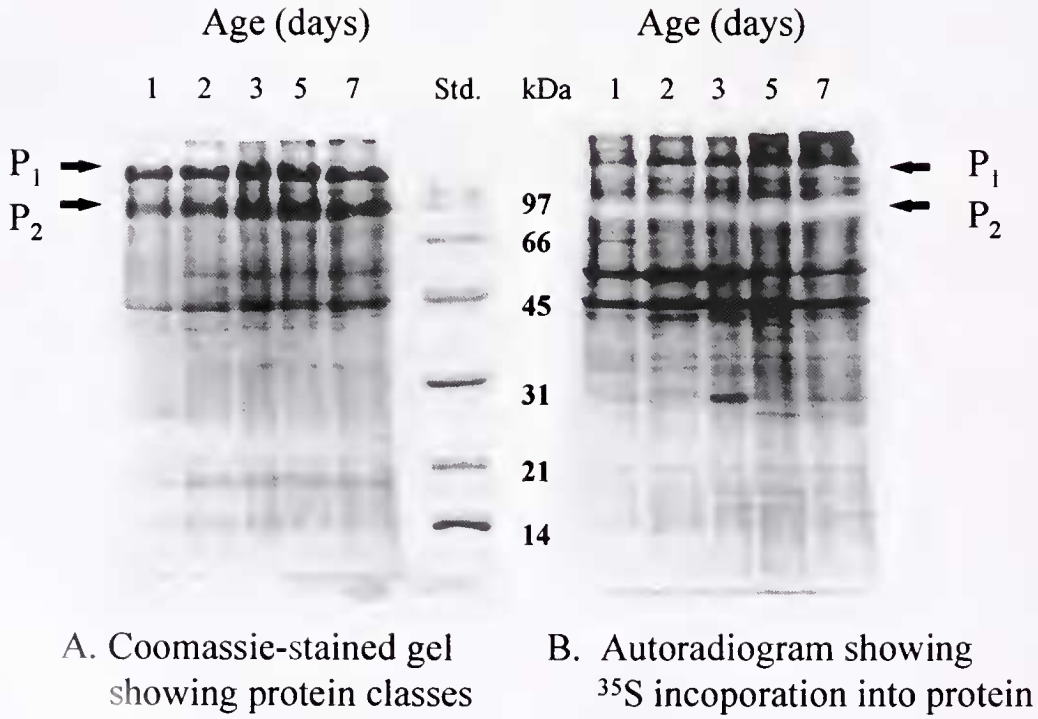

Figure 6. One-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoretic analysis of total soluble proteins in larvae of *Haliotis rufescens*. (A) Coomassie-stained protein pools. (B) Corresponding autoradiogram of proteins labeled with ^{35}S-methionine/cysteine (*i.e.*, newly synthesized proteins). Each lane contains a different larval stage (day 1, 2, 3, 5, and 7) and equal radioactivity (1×10^6 DPM loaded per lane). Bands marked P_1 and P_2 are high-molecular-weight proteins present at each stage of larval development as shown in the Coomassie-stained gel (A) and are not radiolabeled in the autoradiogram (B). Molecular-weight standards are represented with markers from 97 to 14 kDa.

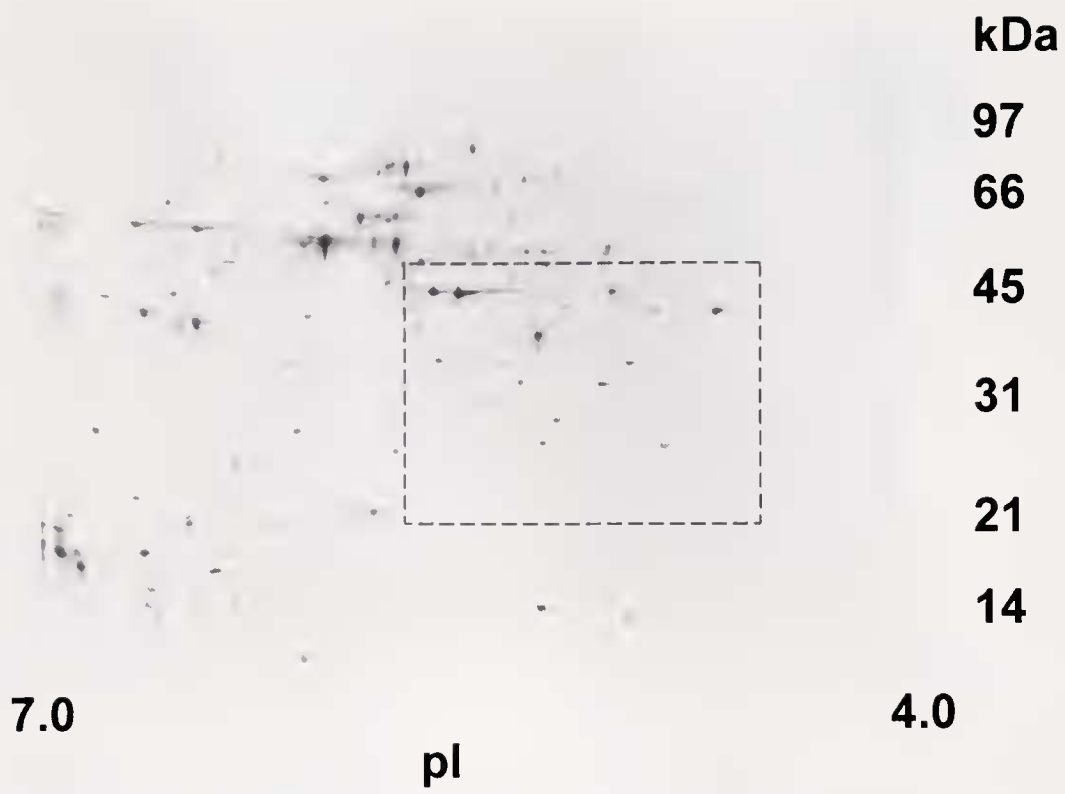

Figure 7. Autoradiogram of proteins in 3-day-old veliger larvae of *Haliotis rufescens* labeled with ^{35}S-methionine/cysteine and separated by two-dimensional gel electrophoresis. Area bounded by the dashed box is displayed in expanded form in Figure 8B. Molecular-weight standards correspond to Coomassie-stained gel (not shown) with markers from 97 to 14 kDa. Isoelectric points (pI) fall within a linear pH gradient from 7.0 to 4.0.

Patterns of protein synthesis

At the analytical resolution of one-dimensional gel electrophoresis, similar patterns of Coomassie-stained proteins were evident during development (1- to 7-day-old larvae). Two classes of high-molecular-weight protein (Fig. 6A: labeled as P_1 and P_2 at 121 and 88 kDa, respectively) dominated the Coomassie-stained protein pool at each larval stage analyzed. The patterns of the existing pool of proteins (Fig. 6A: Coomassie-stained proteins) were different than those of the newly synthesized proteins (Fig. 6B: autoradiogram of labeled proteins), with no detectable synthesis of the two high-molecular-weight protein classes at any larval stage (note lack of signal in Fig. 6B autoradiogram for P_1 and P_2).

The patterns of synthesis of individual proteins were compared, using two-dimensional gel electrophoresis (Figs. 7, 8), for the trochophore stage (1-day-old) and a veliger stage (3-day-old). The two-dimensional separation resolved over 300 proteins that were being synthesized in larvae (Fig. 7). The results of a qualitative analysis (visual absence or presence) are given in Figure 8 to show some of the different proteins being synthesized by 1- and 3-day-old larvae. The main point illustrated in Figures 7 and 8 is that protein synthesis in these larval forms is not limited to any single group (MW or pI) of proteins, but involves many proteins and complex patterns.

Discussion

Larval stages of *Haliotis rufescens* lost protein reserves continuously during development (Fig. 3). By day 8, veligers that were competent to metamorphose contained 34% less protein than the egg. The average daily loss of protein was 21.2 ng protein larva^{-1} day^{-1} (average rate of both cultures, Fig. 3), equivalent to 509 μJ day^{-1} (24.0 kJ g^{-1} protein; Gnaiger, 1983). During this period of protein loss, the absolute rates of protein synthesis decreased fourfold, from 193 to 49 ng protein synthesized larva^{-1} day^{-1} for 1-day-old trochophores and 7-day-old veliger larvae, respectively (Fig. 5; Table II). Note that the rate of protein loss was linear with time (Fig. 3), whereas the decrease in the rate of protein synthesis was nonlinear (Fig. 5). The relationship of these ontogenetic changes in protein loss and synthesis was analyzed further by calculating the fractional rates of protein synthesis during development. When

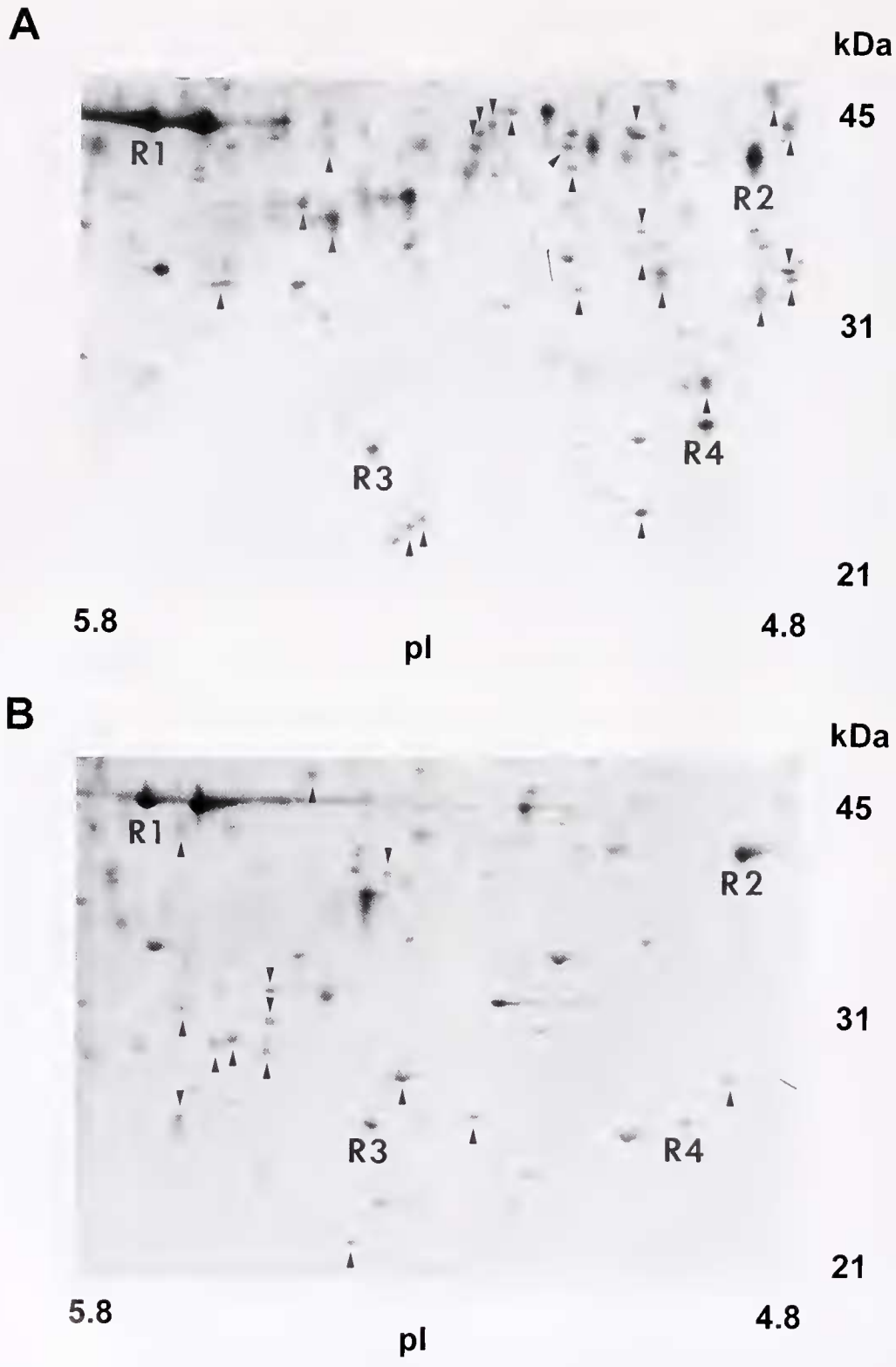

Figure 8. Autoradiograms of two-dimensional separation of ^{35}S-labeled proteins in (A) 1-day-old trochophore larvae and (B) 3-day-old veliger larvae of *Haliotis rufescens* (see Fig. 7 for the entire two-dimensional pattern of protein synthesis for the veliger stage). Each gel was loaded with equal radioactivity (3×10^6 DPM) and exposed to X-ray film for 8 days. Four different proteins that were labeled in each developmental stage are marked as reference points (R1–R4) for comparison of proteins in each gel. Arrows show proteins synthesized at one stage but not the other (by visual comparison of overlaid autoradiograms of trochophore and veliger). Orientation of arrows is not intended to indicate greater or lesser amounts of protein, just presence or absence. Molecular-weight standards correspond to Coomassie-stained gel (not shown) with markers from 45 to 21 kDa. Isoelectric points (pI) fall within a linear pH gradient from 5.8 to 4.8.

expressed as the percentage of a larva's total (whole-body) protein content that was synthesized per day, the fractional rates ranged from 40% (1-day-old trochophore) to 14% (7-day-old veliger) (Table II). These rates of synthesis represent the turnover rates of whole-body protein in larvae of *H. rufescens*. When there is no net deposition (growth) of total protein, as in the lecithotrophic larvae of *H. rufescens*, the rate of protein synthesis is the measure of the rate of protein turnover (Waterlow *et al.*, 1978). Protein turnover, the continual breakdown and replacement of cellular proteins, is a significant component of maintenance metabolism in adult marine invertebrates (Hawkins, 1991). Few data are available in the literature for rates of protein turnover during development of marine invertebrates, making comparisons with our own data difficult. However, Berg and Mertes (1970) measured a rate of protein turnover in sea urchin embryos (*Lytechinus anamesus* at 19°C) of 23% day^{-1}, a value that falls within the range we determined for larvae of *H. rufescens* (14%–40% at 15°C, Table II). These synthesis rates in abalone larvae are not limited to a few specific proteins. The electrophoretic patterns of protein synthesis showed widespread incorporation of ^{35}S-label, indicating a high synthesis rate of multiple proteins (Figs. 6B, 7, 8), as has been found in developing stages of other species of marine invertebrates (*e.g.*, sea urchin embryos: Bedard and Brandhorst, 1983). These complex patterns of synthesis show that many proteins are involved with the synthesis side of the turnover process (synthesis and degradation). The specific proteins that are degraded to provide the precursors for new synthesis have not been identified in this study, but the lack of synthesis of two high-molecular-weight proteins (Fig. 6B: P1 and P2 at 88 and 121 kDa, respectively) suggests that these proteins might be degraded to support the new synthesis of other proteins. Amino acids dissolved in seawater are unlikely to have been a source of precursors for synthesis in our experiments, because the very high numbers of larvae (500 ml^{-1}) that we used would have quickly depleted any substrates present at low concentration in natural seawater.

The energy cost of protein turnover (= synthesis) in larvae of *H. rufescens* can be calculated for comparison with the energy made available from the degradation of protein (Fig. 3). The cost of protein synthesis appears to be constant in different animals and stages of development [*e.g.*, Reeds *et al.*, 1985: mammal = 11.52 ± 1.12 J (mg protein synthesized)$^{-1}$; Hawkins *et al.*, 1989: juvenile marine bivalve = 11.38 ± 8.88 J (mg protein synthesized)$^{-1}$; Vavra *et al.*, unpubl.: veliger larvae of the bivalve *Crassostrea gigas* = 13.2 ± 4.2 J (mg protein synthesized)$^{-1}$]. Using our value for molluscan larvae, the protein turnover rate measured for trochophore larvae of *H. rufescens* of 193 ng protein would equate to a requirement of 2548 μJ $larva^{-1}$ day^{-1}, decreasing in late-stage veliger larvae (7-day-old) to 647 μJ $larva^{-1}$ day^{-1} (turnover rate of 49 ng protein; Table II). The energy gained from the loss of protein (24.0 kJ g^{-1}) during development of *H. rufescens* was constant at 509 μJ day^{-1}. Assuming complete oxidation of the protein depleted, this input of energy from the loss of protein could account for only 20% (509/2548) of the cost of protein turnover in 1-day-old larvae. Obviously, during early development most of the costs of protein turnover have to be supplied from sources other than protein degradation. However, later in development (near metamorphosis) when rates of protein turnover decrease, the energy made available from protein reserves could account for a large percentage (79%) of the costs of turnover.

Protein turnover plays a major role in establishing the metabolic rate and physiological state of animals (Waterlow *et al.*, 1978; Reeds *et al.*, 1985; Hawkins, 1991). For lecithotrophic larval forms, little is known about rates of macromolecular synthesis during nonfeeding development. Our findings with larvae of *H. rufescens* show that these processes proceed at a high rate and undergo dynamic ontogenetic changes, with up to 40% of a larva's whole-body protein being turned over per day compared to depletion rates of only 4%–6%. If abalone larvae are typical of other lecithotrophs, at least in their biochemical and physiological activities, then biosynthetic rates in nonfeeding larval forms may be much higher than expected.

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